risk and this has to be considered. Carefully trained and dependable help must be used, and with such help the risk does not appear to be very great. We have not felt that vaccination of the personnel was necessary.

Some strains of fixed virus injected intramuscularly will cause death in guinea pigs in as high dilutions as the street virus and it is possible that they could be substituted for the latter with satisfactory results.

Maximum immunity develops at 3 weeks, but if more vaccine is injected the interval between the injection of vaccine and of virus can be shortened.

Injury to the tissues by injecting a large volume of virus with considerable force helps in establishing the infection and is responsible for the almost 100 per cent takes in the control animals. This procedure might well be used when testing dogs for immunity.

Conclusion. A simple test for evaluating the antigenicity of rabies vaccine is described and its advantages and disadvantages are discussed.

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Complementary Correction of the Defective Coagulation Mechanism of Hemophilic and Thrombocytopenic Blood.* (17662)

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Evidence has been presented that at least two factors are necessary for the activation of thromboplastin: one contained in plasma and the other supplied by the platelets. According to Quick(1), active thromboplastin is formed through the action of an enzymatic agent liberated by the platelets (thromboplastinogenase) on a plasmatic precursor (thromboplastinogen). A deficiency of thromboplastin capable of severe impairment of the clotting mechanism may be caused either by lack of thromboplastinogen or of thromboplastinogenase, or by the presence in the circulation of an agent capable of inhibiting the platelet factor (antithromboplastinogenase)(2). All 3 conditions have been found in definite clinical states: the first in hemophilia(1), the second in thrombocytopenic purpura(3,4) and the third in some rare types of acquired hemophilia-like disease(2). Thus in hemophilia there is adequate thromboplastinogenase (platelet factor), but a lack of thromboplastinogen(2). In thrombocytopenic blood there is adequate thromboplastinogen, but a lack of thromboplastinogenase. If these assumptions are correct, the addition of hemophilic to thrombocytopenic blood should produce a mixture with a normal clotting mechanism.

We have recently been afforded the unusual opportunity of studying simultaneously a case of classical hemophilia and a 7-year-old girl with a severe thrombocytopenia (9,700 platelets per cm) of unknown cause presenting, as occasionally found in severe purpura(3), a somewhat prolonged clotting time (23 minutes in glass tubes). Blood from the two patients was mixed in various volumetric proportions. Clotting time, percentage of prothrombin activity left in serum, clot retraction and "accelerator effect" of serum were determined in each sample and in the two original bloods.

Methods. Blood was collected from both

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1. Quick, A. J., *Am. J. Med. Sc.*, 1947, v214, 272.

2. Quick, A. J., and Stefanini, M., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 111.

3. Quick, A. J., Shanberge, J. N., and Stefanini, M., *J. Lab. and Clin. Med.*, 1949, v34, 761.

4. Soulier, J. P., *Rév. d'Hématol.*, 1948, v3, 302.

TABLE I.
The Clotting Mechanism of Mixtures of Hemophilic and Thrombocytopenic Blood.

Hemophilic blood, ml	Thrombocytopenic blood, ml	Clotting time, min.	Clot retraction, %	Serum prothrombin activity,* %	"Accelerator effect" of serum, %
2.0	0	42	45	95	6
1.9	0.1	7½	50	80	15
1.8	0.2	6	48	45	18
1.5	0.5	5	44	34	43
1.0	1.0	5	40	25	55
0.5	1.5	5	24	30	37
0	2.0	23	2	90	9

* One hour after completion of coagulation.

patients by venipuncture. A Silicone-coated needle connected with a glass syringe was quickly inserted into a vein and a few ml of blood drawn and discarded. Exchanging this syringe for one coated with Silicone (Dri Film 9987, General Electric Company) and cooled by immersion in an ice bath, 20 ml of blood were withdrawn. The blood was immediately transferred to a Silicone-coated tube kept in an ice bath. Different volumes of hemophilic and thrombocytopenic blood were transferred by means of cooled, Silicone-coated pipettes to Pyrex glass tubes 130 x 10 mm kept in water bath at 37°C. The clotting time of each sample was determined by tilting the tubes every 30 seconds until they could be inverted without spilling the blood. The amount of prothrombin activity left in serum was determined by the one-stage method of Quick(1) according to the technic described by Quick *et al.*(3). The serum prothrombin time obtained was translated into serum prothrombin activity with the use of the dilution curve constructed by the authors(5). The percentage of clot retraction was given as the volume of serum expressed from the clot of 1 ml of whole blood one hour after completion of coagulation (multiplied x 100). The percentage values of "accelerator effect" of serum were determined by the method of de Vries *et al.*(6).

Blood in Silicone tubes from both patients was centrifuged at 800 r.p.m. for 10 minutes to separate the native plasma. The low speed of centrifugation was selected to avoid an excessive loss of platelets by sedimentation. The hemophilic and thrombocytopenic plasmas were then mixed in different proportions. The clotting time, prothrombin activity of serum, clot retraction and "accelerator effect" of serum were determined in each sample, employing the same technics as in whole blood.

Results. Both hemophilic and thrombocytopenic blood gave normal values for fibrinogen, prothrombin and labile factor (factor V, Ac globulin of plasma). Neither contained anticoagulant agents. In both bloods there was evidence of lack of thromboplastin activity as indicated by the high values of prothrombin activity of serum and confirmed by the delayed clotting time and very low "accelerator effect" of serum. When the 2 bloods were mixed in equal volumes, the clotting mechanism became normal (Table I). As the proportion of either blood was increased, the prothrombin activity increased and the "accelerator effect" progressively decreased in serum. However, as little as one part of thrombocytopenic blood in 19 of hemophilic blood reduced the clotting time to normal. Clot retraction, classically defective in thrombocytopenia, became normal when one part of hemophilic was mixed with 3 parts of thrombocytopenic blood. The data presented in Table I are those obtained with mixtures of whole blood; those obtained with native plasma are not given as they were substantially identical.

Discussion. The results presented in this communication support the concept of Brinkhous(7) and Quick(1) that at least 2 factors

5. Stefanini, M. and Crosby, W. H., to be published.

6. de Vries, A., Alexander, B. and Goldstein, R., *Blood*, 1949, v4, 247.

7. Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 117.

are necessary for the activation of thromboplastin: one supplied by the plasma and the other by the platelets. Our results confirm the finding that the platelet factor is quantitatively and qualitatively normal in hemophilia(2) and suggest that the plasma precursor of thromboplastin is normal in thrombocytopenic blood.

A few other data require comment. It will be seen that the addition of 1/20 volume of thrombocytopenic blood to hemophilic blood was sufficient to reduce the clotting time to normal but influenced only slightly the consumption of prothrombin. This indicates that the clotting time of whole blood is less accurate than the prothrombin consumption test as a procedure for the study of hemophilia.

The "accelerator effect" of serum was found to be very low in both hemophilic and thrombocytopenic sera, as previously shown by Alexander and de Vries(8,9). A normal "serum accelerator effect" could, however, be obtained by allowing thrombocytopenic and hemophilic bloods clot in the presence of thromboplastin. In our experiments, the "accelerator effect" of serum appeared to be indirectly proportional to the prothrombin activity of serum.

Summary. In both hemophilic and thrombocytopenic blood the defect of the clotting mechanism reflects a deficiency of active thromboplastin. Addition of thrombocytopenic blood to hemophilic blood results in a mixture in which the clotting time, clot retraction, prothrombin activity and "accelerator effect" of serum all become normal.

These findings support the theory that at least two factors are necessary for the formation of active thromboplastin: a plasmatic agent, deficient in hemophilia, and a platelet factor, defective in thrombocytopenic purpura.

8. Alexander, B. and de Vries, A., *Blood*, 1949, v4, 747.

9. Alexander, B. and de Vries, A., *Blood*, 1949, v4, 752.

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Tuberculin Reaction. IV. Failure of Pyribenzamine to Protect Against Systemic Tuberculin Shock.* (17663)

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The suppressing effect of antihistaminics on allergic reactions involving histamine or histamine-like substances is well established. Although there is no good evidence to indicate that histamine plays a role in the tuberculin reaction, it naturally becomes of interest to inquire whether antihistaminics will suppress the allergic reaction of tuberculin type sensitivity. Information on this point should be helpful in elucidating the mechanism of tuberculin-type reactions as well as being of possible immediate value in the treatment of diseases in which this type of sensitivity is present. These objectives have led to a limited number of investigations on the effect of various antihistaminics on the course of tuberculosis and cutaneous tuberculin sensitivity in experimental animals and man. The conflicting results of the French investigators have recently been summarized by Wissmer(1) who, in his own experiments, observed that oral administration of 500 mg/day of the powerful antihistaminic R. P. 3015 (dimethylaminoethylthio-diphenylamine) did not suppress cutaneous reactivity to tuberculin in either tuberculous or non-tuberculous patients. Sar-

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1. Wissmer, B., *La Revue d'Immunol.*, 1947, v11, 183.